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16. ABSTRACT

The organic components in combustion emissions are composed of thousands of chemicals. Analyzing such a complex mixture for the presence of even a few selected chemicals is difficult and provides information on only a fraction of the chemicals present. Reliance on such limited chemical analysis for determining possible health effects may ignore the contribution of many other chemical components of the effluent. Because combustion emissions are complex mixtures, they have been evaluated as such, rather than by studying a few selected chemicals that might be present. The Salmonella (Ames) assay was used to determine the mutagenicity associated with particles from the effluent of municipal waste combustors, from ambient air collected near a municipal waste combustor, and from the effluent of a pilot-sized rotary kiln in which polyethylene was combusted. Filter samples were extracted with dichloromethane, and concentrated extracts were solvent exchanged into dimethyl sulfoxide for bioassay. Dose-response data were generated, and mutagenicity concentrations (revertants/m^3 of air) or mutagenic potency (revertants/μg of extractable organics) values were calculated.
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USE OF BIOASSAY METHODS TO EVALUATE INCINERATOR EMISSIONS

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ABSTRACT

The products of incomplete combustion from incineration include complex mixtures of organics, particularly polycyclic aromatic compounds, which are present after atmospheric dilution and cooling in emissions as semi-volatile or particulate organic compounds. Combustion emissions are generally recognized as a potential cancer risk since they contain many carcinogenic and mutagenic polycyclic aromatic hydrocarbons. Analyzing such a complex mixture for the presence of even a few selected chemicals is difficult and provides risk information on only a fraction of the chemicals present. Bioassay methods, however, may be directly applied to evaluate the mutagenic and carcinogenic activity of the complex organics from combustion emissions. The Salmonella (Ames) assay was used to determine the mutagenicity associated with particles from the effluent of municipal waste combustors, from ambient air collected near a municipal waste combustor, and from the effluent of a laboratory-scale rotary kiln incinerator simulator in which polyethylene was combusted. Dose-response data were generated, and mutagenicity concentrations or mutagenic potency values were calculated.

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An environmentally acceptable means of disposal of the increasing amounts of municipal garbage has become a major problem for many city managers and engineers. The average citizen now sends 5 lbs of garbage to the dump each day, compared to 2.9 lbs/day in 1960 (1). Another source (2) estimates that this country now generates more than 450,000 tons/day of municipal solid waste. Landfills have been commonly used for disposal of municipal waste; however, in the last 20 years there have been many incidences of soil and ground water contamination. Since 1979, 3500 landfills have closed (1). The siting of new landfills is also becoming difficult in many locations due to shortage of suitable land and increasing citizen resistance to locating landfills in their areas. These problems associated with landfills have provided good incentives to utilize incineration for solid waste disposal. A further major impetus is the economic gain realized thru energy generation during municipal waste incineration (MWI).

This trend toward increased utilization of MWI, however, also mandates the development of scientific techniques needed to ensure protection of the environment and, particularly, the quality of the air that we breathe. Studies have been reported on the analysis of MWI stack emissions but usually for specific compounds or a class of compounds (3,4,5,6). Because combustion emissions or products of incomplete combustion (PICs) can literally consist of thousands of compounds, reliance on a specific analysis of only a fraction of the chemicals present would ignore the contribution of many other potentially hazardous components of the emission. This could result in an erroneous assessment of health risks associated with such emissions. Combustion emissions, therefore, are complex mixtures and this laboratory has chosen to evaluate them as such by using the short-term Ames Salmonella typhimurium bacterial mutagenicity assay (7) to provide a mutagenicity assessment for the whole sample. This assay was chosen because chemicals and mixtures recognized as human carcinogens are generally mutagenic in short-term tests (8) and combustion emissions from other sources have been both mutagenic in this assay and carcinogenic in rodents. It has also been reported that chemicals that are rodent carcinogens across several species and organ sites (trans-species carcinogens) are generally also mutagenic in Salmonella typhimurium (9). The results from studies such as these should ultimately be useful in providing information for the risk assessment of whole incinerator complex mixtures. Studies using the Salmonella assay to characterize MWI emissions have been reported (10,11,12). However, sampling and analytical techniques required for collection and sample preparation of large quantities of stack emissions are in the early stages of development.

This paper presents some of the mutagenicity results from bioassay of dichloromethane extracts of particles and/or semi-volatile organic vapors collected from (a) ambient air monitoring stations located near a municipal incinerator (13), (b) stack emissions from a different municipal incinerator, and (c) a laboratory-scale rotary kiln incinerator simulator during combustion of polyethlyene tubing. Dose-response data are used to calculate mutagenicity concentrations as revertants/m^3 for the ambient study and mutagenic potencies as revertants/μg of extractable organics in the two incinerator emission studies. None of the above field studies have been completed and only a

portion of the results from each are reported here in order to demonstrate the utilization of mutagenicity bioassays.

STUDY METHODOLOGY AND RESULTS

AMBIENT STUDY

Twenty-four hour ambient air samples were collected every 12th day at four monitoring stations located around a modular mass-feed municipal waste incinerator with a burn capacity of 240 tons/day. Incinerator pollution control devices included a 4-field electrostatic precipitator (ESP) followed by a condensing heat exchanger followed by a wet scrubber (packed tower type). One or more of each following type of sampler was located at each sampling site: a) total suspended particulate (TSP) HiVol, used for collection of a mutagenicity bioassay sample on a glass-fiber filter; b) PM10 HiVol, used in part for determination of 0-10 micron particle concentrations for each sampling period; and c) PS-1 sampler, used for simultaneous collection of particles on a glass fiber filter and collection of semi-volatile organics on a polyurethane foam (PUF) plug. One site also served as a QA site and contained two TSP HiVols and two PM10 HiVols. This phase-1 data set represents the first 12 sampling periods between November 5th, 1987 and March 16th, 1988. PUF samples were collected during only one of these periods.

Filter Sample Extraction

The TSP HiVol glass fiber filters were extracted by a dichloromethane sonication procedure (14). Final extracts were concentrated by rotary vacuum evaporation and volumes adjusted to 10 mL. Triple aliquots were used for gravimetric analyses to determine extractable organic mass (EOM) concentrations. An aliquot of the remaining extract was exchanged to DMSO (14) using dry nitrogen evaporation just prior to bioassay.

Bioassay Analysis for Mutagenicity

The Ames Salmonella typhimurium histidine reversion assay (7) with strain TA98 was used for mutagenicity bioassay. Samples having sufficient EOM were tested at a minimum of five doses using triplicate plates with and without Aroclor-induced S9 metabolic activation (+S9 and -S9) at each dose. The +S9 data from January 16th, March 4th, and March 16th were from single plate determinations. Spontaneous reversion rates for TA98 were 25-50 colonies per plate after a 72-hour incubation. A set of positive controls was incorporated in each experiment. These controls included 2-anthramine (0.5 μg/plate) and 2-nitrofluorene (3.0 μg/plate) with and without S9, respectively. A negative control utilized in each experiment consisted of a DMSO blank.

The data were analyzed by the statistical method of Bernstein et al.(15). The slope values are reported as either revertants/μg of extractable organics or revertants/m^3 of air.

Ambient study results

TSP HiVol filters and PS-1 PUF samples from the 12 sampling periods of Nov. 5th thru March 16th were analyzed for mutagenicity. Each sampling period generated five TSP filters and five PM10 mass filters from the four ambient monitoring stations. One TSP filter was received from each of three sites, whereas the QA site generated duplicate bioassay and duplicate PM10 mass filters from the collocated samplers. Data points are missing from the graphs for sample periods for which there were insufficient amounts of extractable organics for both the +S9 and -S9 mutagenicity assays. The January 4th and February 21st TSP bioassay samples were not analyzable due to contaminated S9.

Figure 1 shows the TSP +/-S9 mutagenicity concentrations from each of the sampling periods and compares these data with the particle concentrations obtained from the collocated PM10 HiVol samplers at the four monitoring sites. The two PM10 samplers at the QA site (2nd and 3rd points for each sampling period) showed the highest concentrations of 0-10 micron particles. Likewise, the two TSP samplers from the same site gave correspondingly higher concentrations of both direct-acting (activity in the absence of S9 [-S9] microsomal activation) and indirect-acting (+S9) mutagens. This site is about 0.75 miles to the east of the incinerator and is the farthest from the stack. This site is also closest to the town center and is more heavily impacted by normal city combustion sources. The relative amount of direct-acting mutagenicity in these samples provides further evidence of significant contribution from city sources such as diesel vehicles. It is also interesting to note the general trend in particle and mutagenicity concentrations from all sites was a gradual increase from relatively low levels in November to peak concentrations in mid-January followed by a gradual decrease to low levels in mid-March.

Figure 2 demonstrates the positive correlation between the TSP indirect-acting mutagenicity and the PM10 particle concentrations. The r-squared correlation value of 0.74 would likely be further improved if the identical samplers had been available and used for this comparison.

A set of PS-1 PUF samples from one sampling period was also similarly analyzed to determine relative mutagenicity concentrations resulting from semi-volatile organics. These results (data not shown) from three sites, compared to those for the particle-bound organics, indicate that mutagenicity was associated primarily with the particles. A fourth site, however, indicated a mutagenicity concentration from semi-volatile (PUF) organics that was equal to that obtained from the filter particles. Further studies of the semi-volatile organics are in progress.

MUNICIPAL INCINERATOR STACK SAMPLING STUDY

Incinerator stack emission samples were collected by two prototype dilution samplers. The municipal incinerator used for this initial sampler testing was equipped with an electrostatic precipitator (ESP) control device

only. Each sampler was designed to collect particulate samples in a manner that simulates the flue gas quenching processes occurring upon emission to the atmosphere. A 10:1 dilution with ambient air was used that promotes nucleation of vapor-phase organic compounds and condensation on existing particulate matter. A prototype source dilution sampler (SDS) was designed to collect 10 CFM stack emissions (16) and dilute in a mixing chamber with 100 CFM of heated HEPA- and charcoal-filtered outside air. Diluted particles were collected on a round (approximately 26 inch diameter) Teflon-impregnated, glass-fiber filter. A modified SASS cyclone used at the sampler inlet removed particles >2.5 microns prior to dilution and collection.

A prototype baghouse/dilution tunnel sampler, which was evaluated at the same incinerator, was designed to collect 100 CFM of stack emissions diluted with 1000 CFM of HEPA- and charcoal-filtered outside air (17). The dilute effluent was passed through a baghouse containing a fabric filter cartridge. An air jet above the cartridge was used to provide a reverse pulse of high pressure nitrogen to dislodge particles from the filter for collection in a glass bulb positioned at the bottom of the collector. This sampler was designed to collect the large quantities of sample required for chemical characterization through use of bioassay-directed fractionation and also to generate sample sizes sufficient for mouse skin carcinogenicity studies.

Sample Extraction and Bioassay

The SDS filters were cut into pieces and triple extracted by repeat sonications with dichloromethane. Extracts were concentrated, and EOMs were determined as described previously. Aliquots of remaining extract were concentrated and exchanged to DMSO prior to bioassay.

A 102-gram portion of the baghouse particle sample was Soxhlet extracted with dichloromethane. The extract was concentrated, the percent extractable mass was determined, and the sample was prepared and bioassayed as described previously.

Incinerator Stack Sample Results

Five SDS filter samples were collected during a first sampling study that contained 1.3-3.8 g of particles from 12.8-80.3 m^3 of stack emissions. Sampling period durations ranged from 56 minutes to 360 minutes. The amount of particles collected was, of course, proportional to volume of stack emissions sampled but was also dependent on burn conditions during the sampling period. The percent EOM values averaged 0.87 +/- 0.5. One sample, representing 65.3 mg of extractable organics, was bioassayed. Figure 3 shows little difference between the mutagenic potencies (revertants/microgram of EOM) of the sample in the presence of S9 (26.6 rev/μg) and the absence of S9 (19.7 rev/μg). It is interesting that the sample shows direct-acting mutagenic activity, which suggests the possible presence of direct-acting mutagens such as nitroaromatics. Further studies are in progress to elucidate the presence of such compounds.

A second sampling study, conducted approximately one month after the first study, utilized both the SDS sampler and the baghouse sampler. The purpose of this study was to compare directly the bioassay analyses for samples collected by these two prototype samplers. Water condensation was encountered during this study, however, which prohibited the baghouse sampler from dislodging particles collected on the fabric filter. An acidic wet sample was collected that contained amounts of metal ions resulting from attack on the stainless steel sampler housing by HCl in the stack emission gases. This sample, when bioassayed, proved to be toxic to Salmonella, preventing determination of sample mutagenicity. Extracts from the SDS filters collected during this same study were also found to be toxic in the bioassay system. This result was not expected because the previous mutagenicity analysis of an SDS filter sample had been successful as reported above. This second set of SDS extracts appeared to be more acidic than the first set. This condition likely caused the observed toxicity and demonstrates the need for additional research to design sampling systems for acidic stack gases.

POLYETHLYENE BURN LABORATORY STUDY

In order to study a "worst case scenario," we evaluated the organic extract of particulate emissions from a laboratory-scale rotary kiln incinerator simulator (73 kW; 250,000 Btu/h) in which polyethylene plastic was incinerated under failure mode conditions. The design of the kiln and the conditions under which the plastic was burned have been described (18). Although the kiln was operated at typical incinerator conditions (temperature, excess air, residence time) and the plastic was batch charged into the kiln to examine transient behavior typical of kilns, no afterburner or other pollution control was present on the stack. Thus, the particles obtained represent those produced under less than ideal combustion conditions. The particles were collected using the baghouse/dilution tunnel sampler described above. Particles were extracted, prepared, and bioassayed as described previously.

Figure 4 shows the mutagenic potency (in the presence of S9) of the EOM from the combustion of polyethylene in the kiln to be 18.8 rev/μg, which is similar to that of the effluent from the MWI (Figure 3). However, unlike the MWI effluent, the effluent from polyethylene combustion in the kiln was not mutagenic in the absence of S9 (Figure 4). Such a result is not unexpected because PICs formed during polyethylene combustion contain significant amounts of polycyclic aromatic hydrocarbons (PAHs) (18). These compounds generally require metabolic activation in order to be mutagenic. Thus, it is likely that some of the mutagenic activity observed from combustion of polyethylene in the kiln is due to PAHs or PAH-like compounds.

UTILITY OF BIOASSAY RESULTS

The contrasting results in the absence of S9 between the effluent from a commercially operating MWI (Figure 3) and from a laboratory-scale rotary kiln simulator burning polyethylene plastic (Figure 4) illustrate the utility of

bioassays to help characterize differences between combustion emissions. Fractionation of these organic extracts by means of high performance liquid chromatography and subsequent bioassay of the fractions can be used to identify the major chemical classes that are responsible for the observed biological activity. The coupling of biological analysis with chemical analysis (bioassay-directed chemical analysis) can enhance our understanding of the chemical composition and any consequent health risks associated with incinerator emissions.

The mutagenic potency values, combined with the amount of waste burned, permits calculation of a mutagenicity emission factor. The emission factors could be compared to those from other combustion sources, such as cigarette smoke, coal-fired power plants, wood burning stoves and automobile exhaust to allow incinerator emissions to be put into perspective (19).

SUMMARY AND CONCLUSIONS

The Ames bioassay has been used successfully to determine the mutagenicity of ambient air particles collected near a municipal incinerator. This procedure will be used to provide an assessment of the contribution of the incinerator to mutagen concentrations in air through a) assessment of the relationship, if any, between tons of waste burned and mutagen concentrations at ambient air sampling sites and b) determination and comparison of mutagen concentrations in air samples collected upwind and downwind from the incinerator.

Two prototype municipal incinerator stack samplers have been built, and field testing is currently underway to evaluate their usefulness in: a) assessing the mutagenic potencies of particles and vapor-phase organics emitted by various types of municipal incinerators, b) determining the concentration of mutagens emitted by incinerators, and c) collecting sufficiently large quantities of emission particles that can be used for chemical characterization and carcinogenicity bioassays. Initial testing has encountered problems with corrosive emission gases that have attacked sampling equipment. Some stack sample extracts have also been found to be toxic to Salmonella test organisms and could not be bioassayed without fractionation to separate the toxic components from the mutagenic components. Further modification of sampling equipment and test procedures are currently being explored.

The utility of the Salmonella assay to identify differences among various incinerator emissions will be explored further by fractionating selected emissions and bioassaying the fractions. Such procedures should lead to a better understanding of the chemical composition and possible health risks associated with incinerator emissions.

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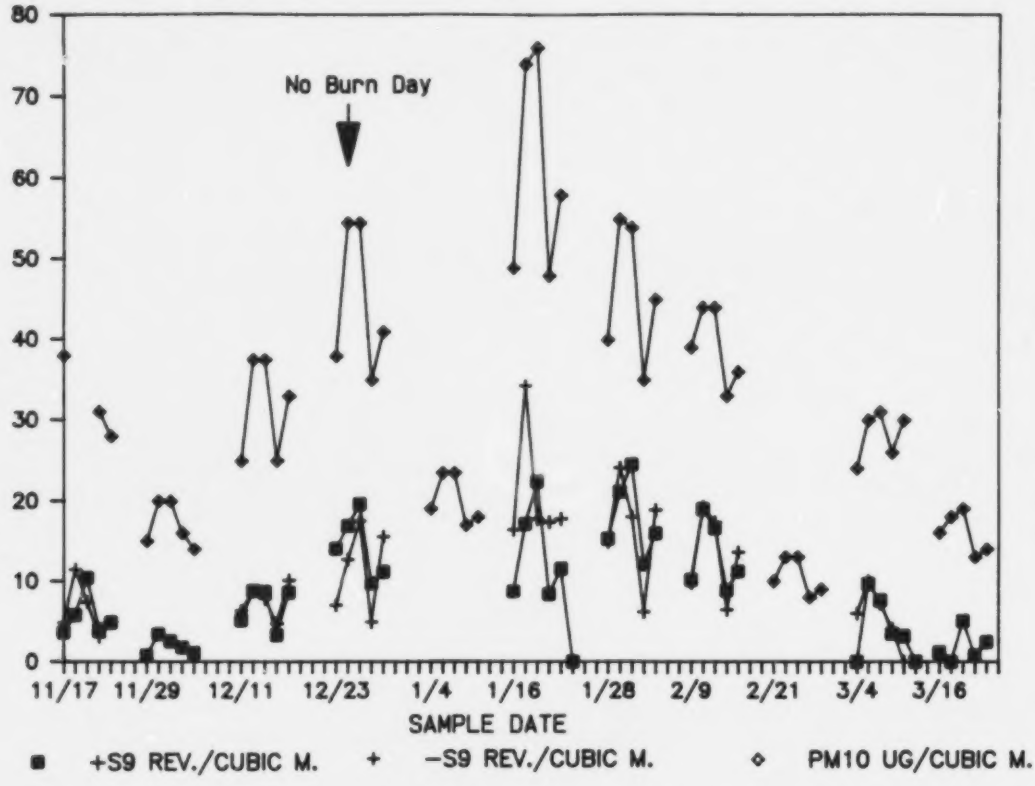

Figure 1. Mutagenicity and Particle Concentrations

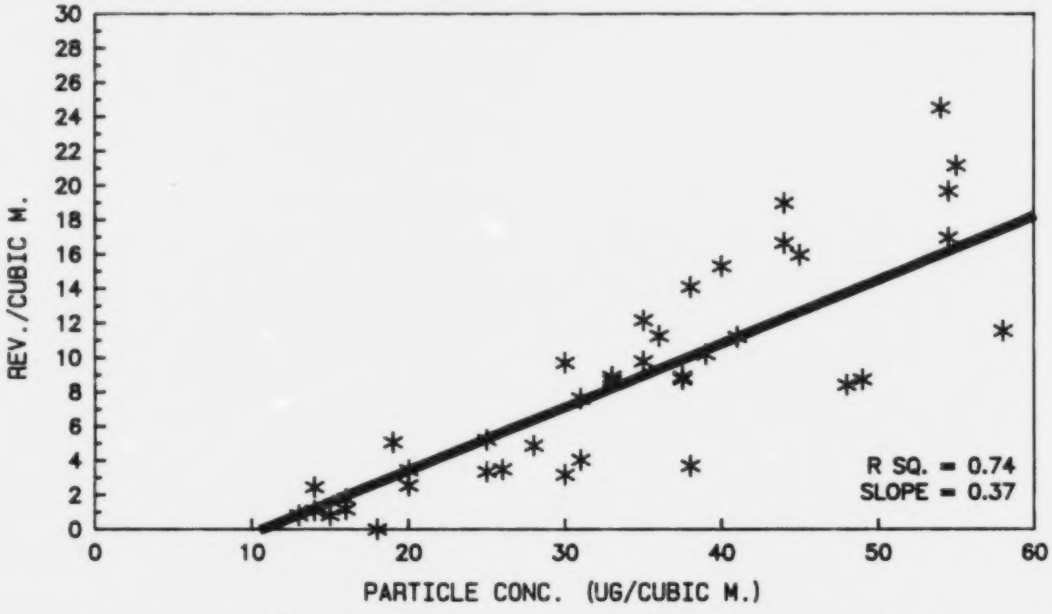

Figure 2. Mutagenicity and Particle Correlation

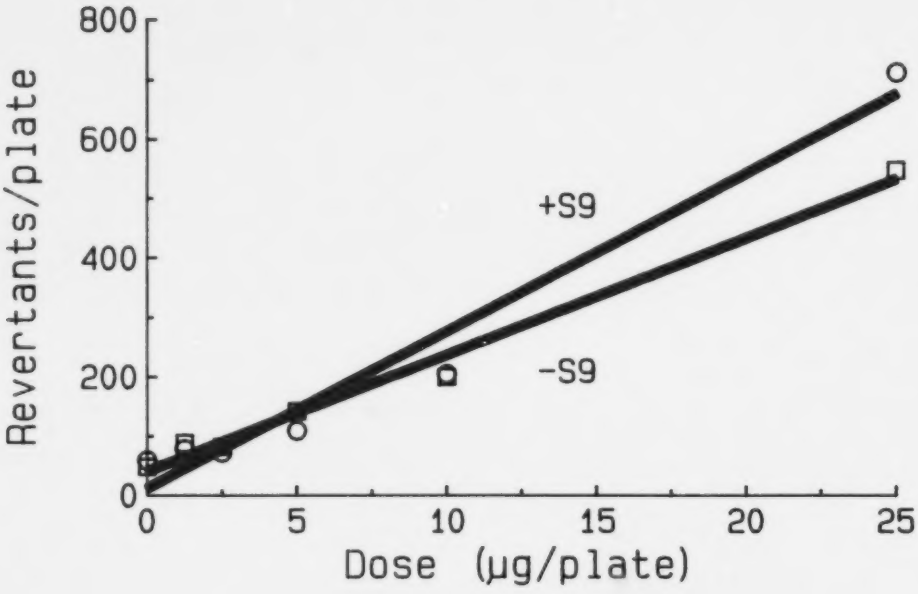

Figure 3. Mutagenicity of MWI Effluent

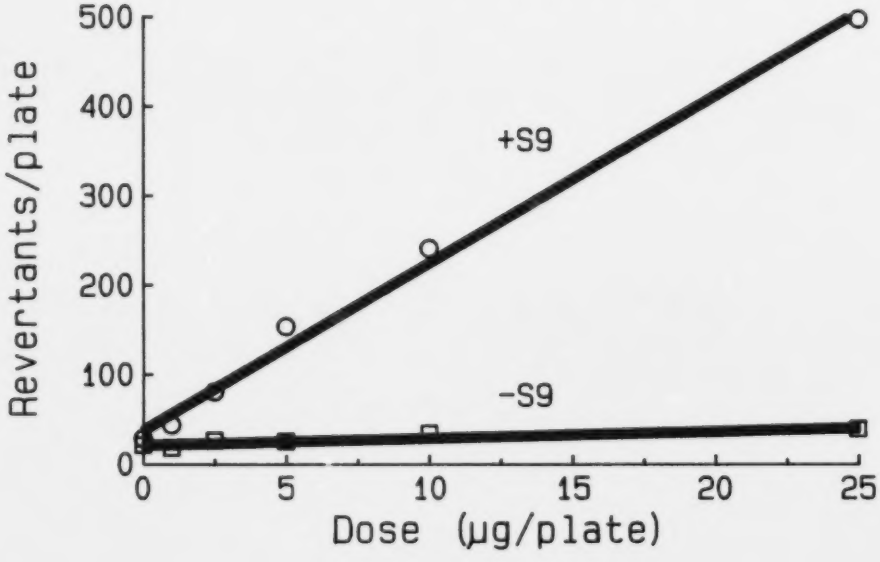

Figure 4. Mutagenicity of Rotary Kiln Effluent